THE EFFECT OF COTTONSEED PRODUCTS AND SELECTED FEED ADDITIVES ON EGG YOLK DISCOLORATIONS

By

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CHAPTER I

INTRODUCTION

Eggs unsuitable for consumption due to a mottled appearance of the yolks have resulted in an economic loss to the poultry industry. Previous work has shown that eggs with mottled yolks are the result of hens being fed cottonseed products or certain feed additives which are believed to cause the discolorations. Considerable research has been done in this area in an attempt to pinpoint the actual cause of yolk discoloration resulting from the incorporation of these ingredients in the layer ration. Many hypotheses have been advanced on this subject, but to date there has been no report of an entirely successful identification and elimination of the cause of the problem.

The purpose of this study was to determine which of the cottonseed products and feed additives were responsible for the discolorations of egg yolks and whether the length of storage period affected the magnitude of the discoloration. Ingredients and additives tested included cottonseed meal, cottonseed oil, pure gossypol, piperazine, dibutyltin dilaurate, vegefat and special beef tallow.

Factors of concern in this study were:

1. determining which of the ingredients caused yolk mottling,
2. determining the effect of storage temperature on yolk mottling of eggs from hens fed the tested ingredients, and

3. comparing the mottling as well as other yolk abnormalities among eggs produced by hens fed the various ingredients.

It is hoped that the data presented herein will be of consequence in the effort to identify and eliminate the cause of yolk discolorations and defects in chicken eggs, and in so doing, aid in the elimination of the economic loss to poultryman which results from these abnormalities.

CHAPTER II

REVIEW OF LITERATURE

The successful incorporation of cottonseed products in a layer ration has, for some time, been a problem for nutritionists as well as for poultrymen. Evidently, the first report of the problem was by Roberts and Rice (1891). They fed rations containing cottonseed meal which produced mottled yolks. However, no effort was made to determine the real cause of the abnormality. Similar results were indicated by Lamon and Lee (1917), who found that the "greenish spots on the yolks" were more frequent during the warmer portion of the year.

A report by Thompson et al. (1930) showed olive yolks being produced from hens fed a diet containing cottonseed meal. Not all eggs produced were found to be objectionable and some eggs retained their normal color regardless of the amount of cottonseed meal tested. These authors conducted tests to determine whether gossypol might be the causative agent, and the results indicated that it had no part in the production of the mottled yolks.

Similar discolorations, with some increase in mottling due to storage of eggs, were reported in the New Mexico Agriculture Experiment Station 38th Annual Report. Walker et al. (1929) reported that storage increased mottling, with some eggs becoming almost black, and the work of Kempster (1930) supported these earlier findings. Sherwood (1928) reported discolored yolks from cottonseed meal rations only after the

eggs had been in cold storage for four weeks. In a later study, Sherwood (1931) showed that discoloration increased with increased levels of cottonseed meal in the diet beginning with two grams of meal per hen per day. Due to a comparison of results from several cottonseed products, the substance causing the discoloration was believed to be in the cottonseed oil or something closely related to it. Olive to brown-colored yolks resulting from the feeding of cottonseed meal were reported by Schaible et al. (1946). Not all eggs from the cottonseed meal ration showed the characteristic discolorations.

Kurken et al. (1948) found that no objectionable egg yolks resulted from feeding isopropanol extracted meal, but hydraulic process meal did produce discolored yolks. Somewhat similar results were stated by Halloran and Cavanagh (1960), in that properly treated and processed cottonseed meal could be included in the layer ration at levels up to ten per cent of the total ration. Their results also showed that higher storage temperatures adversely affected the color of egg yolks from hens fed cottonseed meal rations. These findings supported those of Lamon and Lee (1917).

Upon testing cottonseed meal produced from glanded and glandless cottonseed, Heywang et al. (1965) found that meal from glanded cottonseed produced a high incidence of mottled yolks while that from the glandless seed produced only limited mottling and in some cases no mottling. Hexane extracted meal produced the largest number of mottled eggs of any of the processing methods studied. This report confirmed earlier work by Stephenson and Smith (1952), which showed 100 per cent mottled yolks from solvent extracted meal. In this latter study, screw

pressed cottonseed meal was found to be as desirable as soybean oil meal for the poultry ration.

Heywang (1957) showed that the ammonia test, proposed by Schaible et al. (1934), was not an adequate indication of which eggs would show discolorations after storage. Heywang et al. (1961) suggested that neither the ammonia test nor the Available Gossypol Units Test proposed by Grau (1958) was able to predict which eggs would show yolk discolorations after storage. Heywang et al. (1961), however, did find that the ammonia test and the halphen test would give an accurate prediction of mottling after storage.

Thompson et al. (1930) found no evidence to show that gossypol was the factor involved in the discoloration of yolks of eggs from hens being fed rations containing cottonseed meal. Contradictory results by Schaible et al. (1934) indicated that gossypol was the cause of the discoloration, since the compound was shown to be present when mottling occurred and absent when mottling failed to occur. Discolorations caused by gossypol were reported by Lorenz (1939) and by Swensen et al. (1942) in that the feeding of free gossypol produced the same results as cottonseed meal, and they suggested that ferric iron combined with the gossypol to produce the characteristic color. It was interesting to note that the observation was also made that the addition of ferric salts to the diet proved effective in the prevention of the discoloration.

Woronick and Grau (1954) reported an absorption spectra of 380-400 mμ for a yellow component associated with the cephalin fraction of egg yolks from hens on a cottonseed meal ration. Gossypol has a peak at 365 mμ and was believed to be the yellow pigment.

Heywang (1947) found that cottonseed meal with a free gossypol content as low as .059 per cent produced discolored yolks, and later results (Heywang et al., 1955) showed that the percentage of yolk discoloration and the degree of discoloration increased as the level of free gossypol in the diet was increased. Individual hen differences, with respect to their ability to produce mottled yolks, were also found. Rations containing as low as .00025 per cent of free gossypol were shown to produce egg yolk discolorations, (Heywang, 1957). Heywang et al. (1962) noted that dark discoloration could be prevented by oil dipping of eggs from hens on a diet containing less than .008 per cent of free gossypol. Spraying with oil also proved effective in preventing dark discolorations in yolks from hens on a ration having .001-.002 per cent of free gossypol.

Kemmerer et al. (1961) indicated that the discolorations attributed to gossypol from cottonseed meal were not due to free gossypol alone, but to a combination of gossypol and cyclic fatty acids such as sterculic and other acids with similar structures.

Salmon colored egg yolks have been reported to result from the use of cottonseed oil in the layer ration. Schaible and Bandemer (1946) suggested that this could be the result of the diffusion of the conalbumen fraction of the egg white into the yolk and its combination with iron. Heywang et al. (1954) reported results which described egg yolks as orange in color.

Salmon-pink colored yolks resulted from the feeding of cottonseed oil (Pepper et al., 1962). None of the eggs could be classed as mottled when the oil alone was used in the ration; but when acidulated soapstock was included, mottling appeared. It was noted that gossypol, added at

a level comparable to that received in the oil, produced no mottling. Lower storage temperatures were shown to accentuate the yolk abnormalities.

The fact that cottonseed oil at the 2.5 per cent level produced salmon colored yolks was found by Evans et al. (1957); however, fewer olive yolks were found in eggs from hens fed the cottonseed oil ration than in those from hens fed the ration containing cottonseed meal. Another report by Evans et al. (1960) showed that the eggs from hens fed the cottonseed oil rations not only had salmon to brown yolks but that they also were very viscous when examined.

Feeding rations containing either Sterculia foetida seeds or crude cottonseed oil to laying hens caused them to lay eggs which had yolks that contained an increased proportion of saturated fatty acids and a decreased proportion of monoenoic acids. Over 50 per cent of the fatty acids in egg yolks from hens fed cottonseed oil or S. foetida seeds were saturated (Evans et al., 1961a). As a result of increasing the saturated fatty acid content of the egg yolk, the solidification point was increased. This caused egg yolks to be less fluid at cooler temperatures. Evans et al. (1962) found similar results when examining ovaries from hens fed S. foetida seeds or cottonseed oil. The saturated fat content was higher than from a normal ration. Although no actual egg yolks were examined, nothing indicated that eggs would differ from the ovaries.

These reports and those of other researchers offer an explanation to results reported by Shenstone and Vickery (1959). The eggs from hens fed sterculic acid, which were examined in the study, were described as pasty when cold. Some of the yolks were so severely affected that they

could be described as spherical when placed on a flat plate and could be picked up between the fingers without rupturing the vitelline membrane.

In an effort to study the effect of heat on the cottonseed oil, Evans et al. (1960) found that oil heated at 240° C. for one hour would not yield a positive halphen reaction or the discolorations previously mentioned. However, heating for lesser amounts of time and at lower temperatures would produce discoloration as well as a negative halphen test. Evans et al. (1961b) further observed salmon colored eggs from hens fed cottonseed oil rations; however, in this experiment, one of the hens failed to exhibit the defect.

Kemmerer et al. (1962) noted that the addition of crystalline gossypol to rations containing crude cottonseed oil intensified the discolorations caused by the gossypol in eggs stored for periods of one to three months at 0° C. Kemmerer et al. (1963) further reported that .2 per cent of cottonseed oil combined with 3 mg. per day of pure gossypol resulted in discolored yolks, while .1 per cent of cottonseed oil with the same level of gossypol had no effect.

Ellis et al. (1931) reported cottonseed oil ingested by hogs to raise the saturated fat content of body fat. Brown et al. (1963) showed an increase in the saturated fatty acid content of milk as a result of feeding cottonseed oil. From these findings it would seem that beef tallow could be expected to contain high amounts of saturated fatty acids as a result of the animal being fed cottonseed oil.

Mottled or discolored yolks have been reported as the result of the use of the feed additives piperazine and dibutyltin dilaurate in the layer ration. It is difficult to determine exactly whether these reports relate to the same kind of mottling as is attributed to cottonseed

meal. It may be that these discolorations could better be described as yolk defects.

Piperazine was reported to be effective in eliminating A. galli from infected poultry by Edgar et al. (1957). Fry and Wilson (1967) found that piperazine did not significantly affect yolk mottling. Contradictory results showing that egg yolk discolorations were found when piperazine was incorporated into the layer ration were reported by Beane et al. (1965). Warm weather increased mottling as did storage in this particular study. Similar results were mentioned in a report by Peardon et al. (1965). Again, high environmental temperature increased discoloration.

Edgar (1956) found that another compound, dibutyltin dilaurate, was effective in the removal of R. cesticillus from poultry. Peardon et al. (1965) reported a high incidence of interior anomalies in eggs from hens on a diet containing double the recommended levels of piperazine, phenothiazine and dibutyltin dilaurate as a triple combination. Piperazine was the only compound to be tested singly. These anomalies included both blood spots and mottling. Fry and Wilson (1967) found that dibutyltin dilaurate, singly or in combination with piperazine or phenathiazine, had a detrimental effect on the yolk coloration.

No indication as to the day-to-day effect of storage has been found in literature. Nothing as been found in literature concerning the use of beef tallow which gives a positive halphen test as a result of the animals being fed cottonseed meal at high levels. Comparisons have not been made concerning this kind of tallow, dibutyltin dilaurate and cottonseed products. The purpose of this study is to describe the relationship of the tallow and dibutyltin dilaurate as compared to

cottonseed products and to report on the effect of these and other feed ingredients on the yolks of stored chicken eggs.

CHAPTER III

MATERIALS AND METHODS

The study reported in this thesis was concerned with the effect of cottonseed products and selected feed additives on yolk discoloration in chicken eggs.

Eggs used in this experiment were obtained from 112 commercial hybrid White Leghorn hens. The birds were confined in cages in a windowless, environment controlled house on the Oklahoma State University poultry farm. Due to the windowless house, continuous artificial light was supplied for fourteen hours each day.

Eggs were collected each afternoon after 4:30 p.m., identified as to hen and date of oviposition, held overnight at 12° C., and then placed in storage at either 2° C. or 25° C. for periods ranging from one to thirty days. No attempt was made to control humidity at either of the storage locations. Upon completion of the storage period, the eggs were removed from storage, broken and the yolks subjected to a visual examination. An egg breaking table was used which incorporated a mirror allowing simultaneous examination of the top and bottom of the yolk. An indication of the severity of mottling was obtained by assigning the numbers 0, 1, 2, or 3 to each individual yolk. Eggs receiving the "0" score were normal eggs, "1" indicated some slight mottling, "2" indicated large areas of mottling, and "3" was used to indicate that the entire yolk exhibited a mottled appearance. The term mottling was

given to the olive or brownish colored areas on the egg yolks. All eggs receiving a score of "1" might not have been considered objectionable by the consumer, but they would not be considered completely normal. All eggs receiving the rating of "2" or "3" would certainly have been objectionable from a consumer standpoint. Other defects such as yolk color, viscosity, and blood spots were noted and recorded.

Two eggs from each hen for each storage period at each temperature were observed. In addition, two eggs from each hen were examined the day they were laid in order to record the characteristics of fresh eggs. A total of 13,664 eggs from 112 hens were used in the final analysis.

Prior to starting the hens on the experimental rations, all hens were fed rations similar to the control ration to insure that no cottonseed products or miscellaneous feed additives were consumed before the test rations were begun.

The ration was a practical-type layer diet formulated by the Poultry Science Department of Oklahoma State University. Substitutions and additions were made in order to incorporate the desired ingredients into the rations. The complete ration and the amount of each of the test ingredients are shown in Appendix A and Appendix B.

In an attempt to identify gossypol in the yolk of the mottled eggs, several tests were conducted on an acetone powder prepared from egg yolks. The tests included:

1. ammonia;
2. methyl-ethyl ketone and oxalic acid, plus aniline and chloroform; and
3. chloroform and antimony trichloride.

The amount of total gossypol present in the ration was determined by the method suggested by the American Oil Chemists Society. Results indicated that the test was not sensitive enough to give accurate results concerning any ration except ration 1. When the control ration was used as a blank, the analysis indicated that ration 1 contained 0.031 per cent gossypol.

The design of the experiment is shown in Figure 1. After obtaining observations from all storage periods, the data were analyzed using the facilities of the Statistics Department on the Oklahoma State University campus. The analysis presented in this thesis was obtained through the use of the 7040 computer system presently employed by the Oklahoma State University computing center.

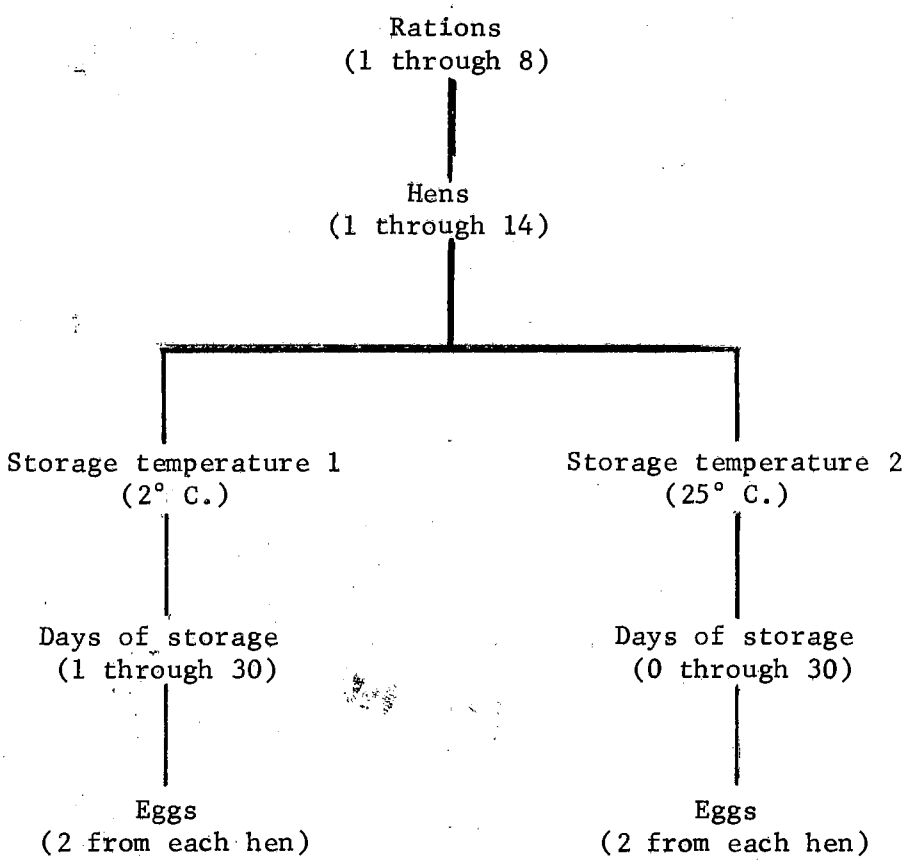

Figure 1. Experimental Design for the Study

CHAPTER IV

RESULTS AND DISCUSSION

The purpose of this experiment was to determine the day-to-day effect of cottonseed products and various selected feed additives on stored chicken eggs. Two storage temperatures, 2° C. and 25° C., were used and the storage periods were from one to thirty days. The tested ingredients included: cottonseed meal, cottonseed oil, piperazine, dibutyltin dilaurate, special beef tallow, vegefat (methyl ester), and pure gossypol.

The results presented herein will be discussed under headings corresponding to the above listed ingredients.

Tables used to explain results will include defects defined as: (1) mottled yolks; (2) blood spots; (3) yolk condition, indicating the viscosity of the yolk; and (4) abnormal color, indicating lighter or darker than normal areas on the egg yolks. These data from each ration include observations from 840 eggs stored at temperature 1 and 868 eggs stored at temperature 2. The difference in numbers of eggs at the two temperatures stems from the fact that eggs broken the day of oviposition (zero days of storage) have been included in the results reported for temperature 2.

Table I shows the observed results from all rations. Mottling was observed in a few eggs from all rations, but the eggs from ration 1 showed the highest degree of mottling. Analysis showed that 37.14

TABLE I

A SUMMARY OF THE TOTAL ABNORMALITIES OBSERVED IN ALL EGGS AT EACH STORAGE TEMPERATURE[1]

Temperature 1 (2° C.)

Ration	Mottled Yolks	Blood Spots	Viscous Yolks	Other Discolorations
1	37.14	00.24	00.48	00.60
2	00.12	00.36	68.93	14.52
3	00.12	00.00	00.60	00.36
4	01.90	05.95	00.60	26.43
5	02.26	00.24	00.71	01.79
6	01.31	00.95	00.00	00.83
7	00.00	00.95	00.71	00.48
8	01.31	00.83	00.36	00.60

Temperature 2 (25° C.)

Ration	Mottled Yolks	Blood Spots	Viscous Yolks	Other Discolorations
1	40.09	00.69	00.00	02.07
2	00.35	00.35	00.00	16.36
3	00.46	00.81	00.00	06.34
4	03.46	05.53	00.00	46.19
5	02.76	00.35	00.00	09.45
6	01.38	00.35	00.00	02.88
7	00.12	01.04	00.00	06.45
8	00.81	01.27	00.00	05.41

[1]Figures are in terms of percentage of total eggs broken.

per cent of the eggs stored at temperature 1 (2° C.) and 40.09 per cent of the eggs stored at temperature 2 (25° C.) exhibited some evidence of a defect that could be called yolk mottling. All rations other than ration 1 failed to produce mottled eggs to an extent that would create any economic problem. The range among the other rations was from zero to 2.26 per cent of the eggs showing the defect.

The occurrence of blood spots was noted, but only in ration 4 was there any noticeable difference among the rations. This was due to one particular hen which produced an abnormally large number of these blood spots throughout the test period.

Other yolk defects, classified here as discolorations, were found in eggs among all of the rations. It was rather evident that this defect was more a result of storage temperature differences than of ration. As examples of this, it can be seen that for ration 2, 14.52 per cent and 16.36 per cent discolorations were found for storage temperatures 1 and 2, respectively. An even more dramatic result can be seen for the eggs produced from ration 4, in which the percentage of discolorations increased from 26.43 per cent for temperature 1 to 46.19 per cent for temperature 2. Although discolorations were observed among the other rations, the low percentages make it difficult to attribute them to ration composition. Even the control ration (ration 7) produced some eggs with this defect, indicating that there was probably a tendency for the individual making the observations to be overly conscious of this phenomenon.

Ration 7 (control) produced eggs with 6.45 per cent from temperature 2 showing the discoloration as compared to 0.48 per cent from temperature 1. This indicated that storage temperature is important in

the prevention of this defect and that temperature is the causative agent rather than the tested ingredients.

In an effort to ascertain whether or not there were differences among hens in the production of mottled yolks, Friedman's rank test was applied to the data (Siegel, 1956). The results of that test are shown in Table II. These results show that only in ration 1 were there significant differences among hens. This was, no doubt, due to the fact that only from ration 1 was there any appreciable number of mottled eggs produced.

TABLE II

CHI-SQUARE VALUES OBTAINED FROM THE STATISTICAL ANALYSIS OF DATA ON MOTTLED YOLK PRODUCTION

Ration	Temperature 1 (2° C.)	Temperature 2 (25° C.)
1	176.44**	143.88**
2	0.08	0.21
3	0.08	0.43
4	1.60	3.71
5	5.52*	2.08
6	2.08	2.32
7	0.00	0.08
8	3.08	0.85

*Significant at the level less than .98

**Significant at the level less than .001

Cottonseed Meal

The egg yolk defects produced as a result of incorporating 9 per cent of cottonseed meal, containing 0.031 per cent of gossypol in the layer ration, are shown in Table III. Those results show that 37.14 per cent of the eggs stored at temperature 1 and 40.09 per cent of the eggs stored at temperature 2 were found to contain mottled yolks. The other abnormalities observed were not exhibited frequently enough to be of economic significance. The percentage of mottled eggs included all eggs other than those considered completely normal. Many of these eggs ranged in color from olive-brown to almost black.

TABLE III

PERCENTAGES OF EGG YOLK ABNORMALITIES OBSERVED AMONG EGGS PRODUCED FROM RATION 1

Abnormality	Temperature 1 (2° C.)	Temperature 2 (25° C.)
	Per Cent	
Mottled yolks	37.14	40.09
Blood spots	00.24	00.69
Viscous yolks	00.48	00.00
Other discolorations	00.60	02.07

A graphic representation of the occurrence of mottled yolks from ration 1 is shown in Figures 2 and 3. These graphs show the percentage of mottled and non-mottled eggs observed at each of the storage times and both storage temperatures. The trend in the occurrence of mottled yolks appears to have increased slightly during the first eight days of storage and then remained relatively constant for the remainder of

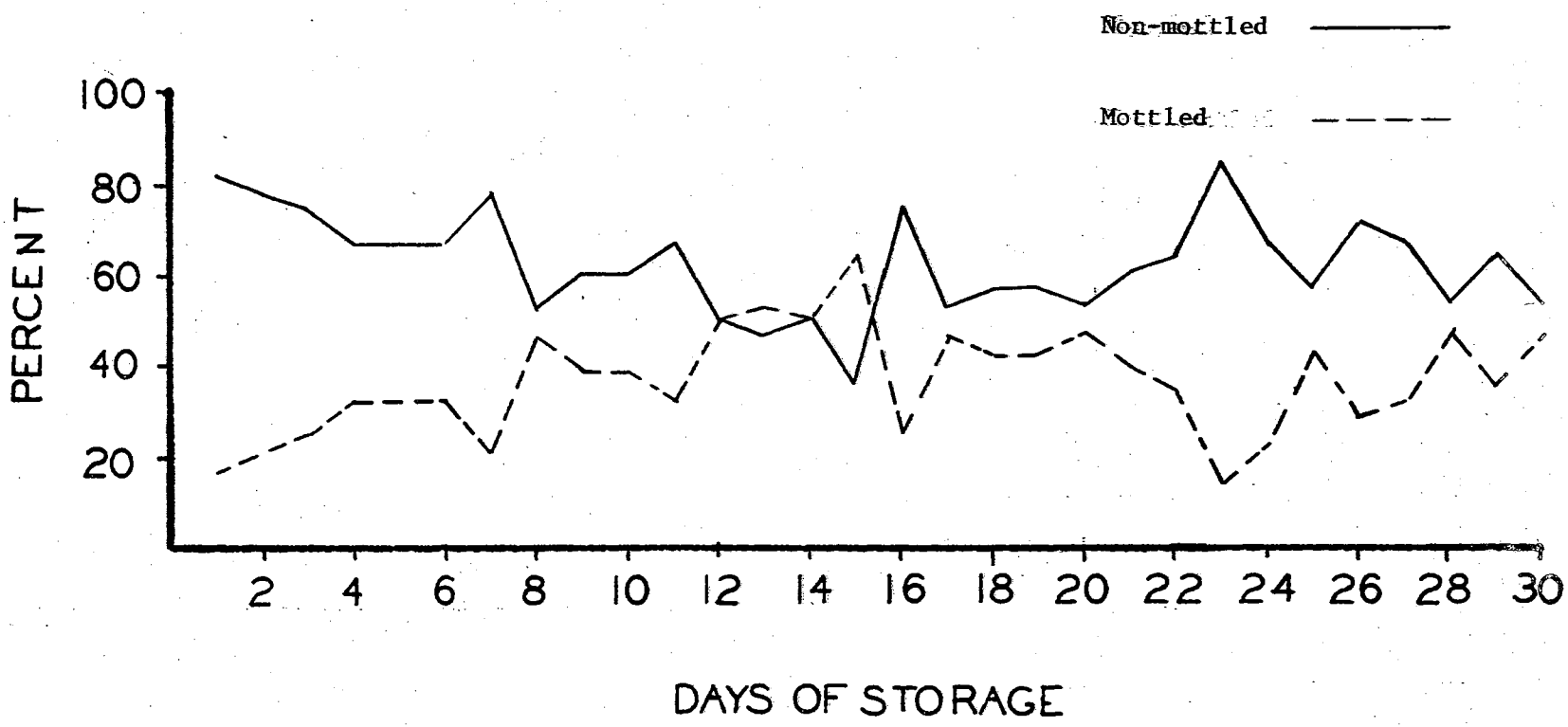

Figure 2. Percentages of Mottled and Non-Mottled Eggs Observed From Ration 1 After Storage at Temperature 1 (2° C.)

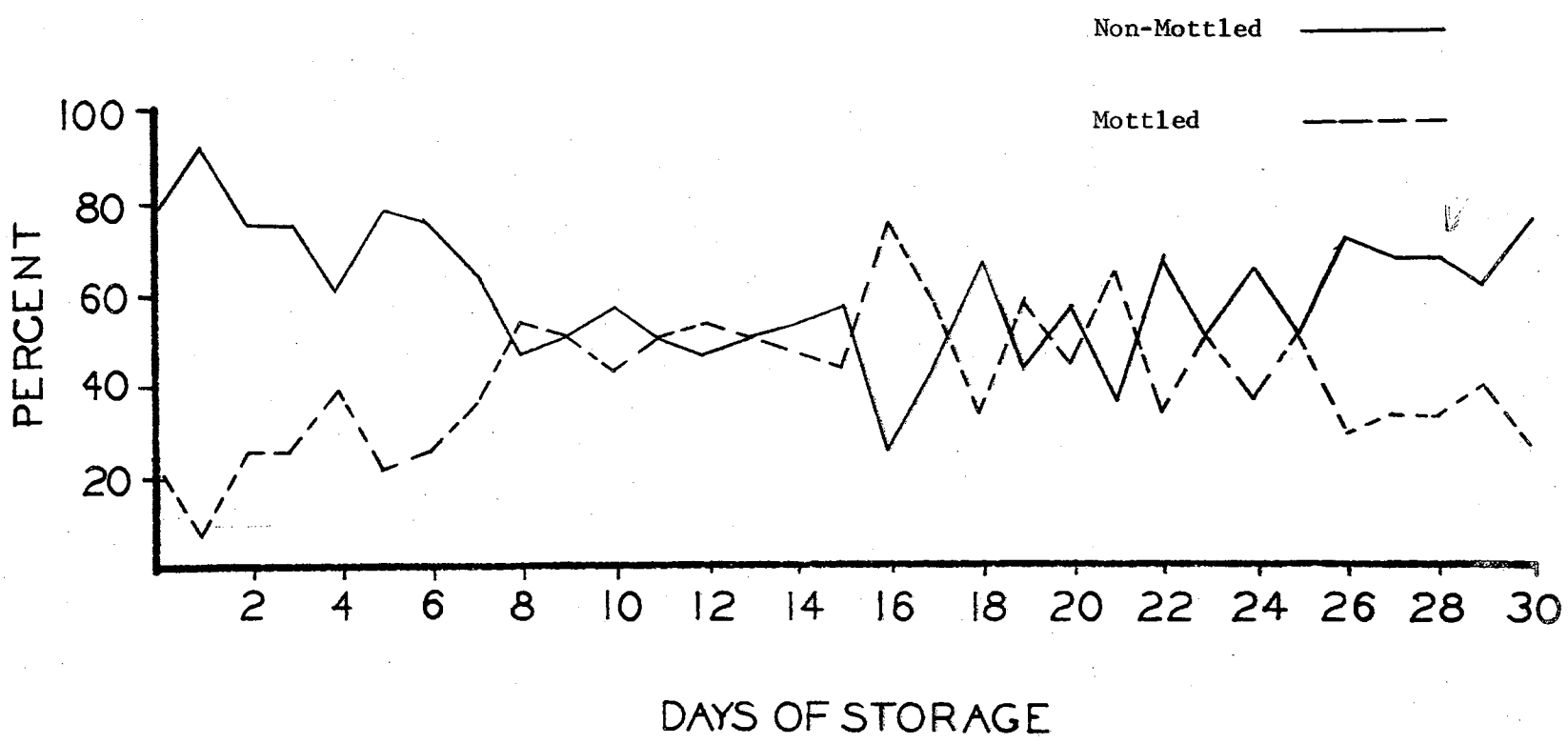

Figure 3. Percentages of Mottled and Non-Mottled Eggs Observed From Ration 1 After Storage at Temperature 2 (25° C.)

the storage period. This was true for both temperatures. Eggs broken on the day of oviposition were recorded under temperature 2, which accounts for the fact that there are no observations listed for day zero at temperature 1. A complete listing of the observations relative to egg yolk mottling by days of storage is shown is shown in Table IV. As can be seen from the table, the percentage of mottled eggs varies greatly through the storage periods, with no definite pattern being shown in any of the observations. In eggs from temperature 1, the percentage of non-mottled eggs ranges from 85.72 at day 23 to 35.71 at day 15. At temperature 2 the range is from 92.85 at day 1 to 25.00 at day 16. This variation shows that mottled eggs are just as likely to appear at the beginning of the thirty day period as at any other period. Earlier reports in the literature had shown an increase of mottling with an increase of storage time; however, in most of these studies, the length of storage mentioned was in excess of the 30 days reported here. Today's commercial marketing procedures in which the product is in the consumer's hands within 30 days, was used as a basis for limiting the storage in the present study.

Records were kept on each individual hen and a study of the data revealed large differences in the percentage of mottled eggs produced by each hen. Mottling was evident in almost all of the eggs from some hens and absent in practically all of the eggs from others. From the total of 14 hens on ration 1, two hens (numbers 1 and 13) were selected to represent the extremes in mottled yolk production. The data from these two hens are illustrated in Table V.

It can be readily seen from the table that of the eggs produced by hen number 1, 88.4 per cent from temperature 1 and 90.3 per cent from

TABLE IV

PERCENTAGE OF TOTAL EGGS CLASSIFIED BY MOTTLING SCORES ACCORDING TO STORAGE TEMPERATURE AND DAYS OF STORAGE

Days of Storage	Temperature 1				Temperature 2			
	Mottling Score				Mottling Score			
	0	1	2	3	0	1	2	3
	Per Cent							
0	00.00	00.00	00.00	00.00	78.57	07.14	10.71	03.57
1	82.15	03.57	14.28	00.00	92.85	00.00	03.57	03.57
2	78.57	00.00	21.43	00.00	75.00	07.14	14.28	03.57
3	75.00	07.14	10.71	07.14	75.00	07.14	14.28	03.57
4	67.86	07.14	21.43	03.57	60.73	21.42	10.71	07.14
5	67.86	14.28	14.28	03.57	78.57	14.28	07.14	00.00
6	67.86	17.85	10.71	03.57	75.00	03.57	17.85	03.57
7	78.57	10.71	07.14	03.57	64.29	10.71	17.85	07.14
8	53.57	21.43	21.43	03.57	46.42	21.43	25.00	07.14
9	60.73	21.43	07.14	10.70	50.00	35.72	10.71	03.57
10	60.73	17.85	17.85	03.57	57.15	21.43	17.85	03.57
11	67.86	17.85	10.71	03.57	50.00	14.28	25.00	10.71
12	50.00	35.72	10.71	03.57	46.42	35.72	17.85	00.00
13	46.42	35.72	14.28	03.57	50.00	28.57	14.28	07.14
14	50.00	32.14	10.71	07.14	53.57	28.57	10.71	07.14
15	35.71	32.14	21.43	10.71	57.15	35.72	07.14	00.00
16	75.00	07.14	14.28	03.57	25.00	42.86	32.14	00.00
17	53.57	35.72	07.14	03.57	42.86	42.86	10.71	03.57
18	57.15	28.57	07.14	07.14	67.86	10.71	14.28	07.14
19	57.15	28.57	14.28	00.00	42.85	35.72	14.28	07.14
20	53.57	28.57	14.28	03.57	57.15	21.43	14.28	07.14
21	60.73	21.43	17.85	00.00	35.72	35.72	25.00	03.57
22	64.29	21.43	07.14	07.14	67.86	14.28	17.85	00.00
23	85.72	10.71	00.00	03.57	50.00	25.00	21.43	03.57
24	67.86	10.71	21.43	00.00	64.29	14.28	17.85	03.57
25	57.15	32.14	10.71	00.00	50.00	35.72	07.14	07.14
26	71.43	17.85	10.71	00.00	71.43	10.71	10.71	07.14
27	67.86	17.85	10.71	03.57	67.86	14.28	17.85	00.00
28	53.57	21.43	21.43	03.57	67.86	14.28	14.28	03.57
29	64.29	21.43	14.28	00.00	60.73	21.43	10.71	07.14
30	53.57	25.00	17.85	03.57	75.00	07.14	17.85	00.00

temperature 2 were given mottling scores of either "2" or "3." In contrast to this there were no eggs from hen number 13 stored at temperature 1 and only 1.6 per cent stored at temperature 2 classified into these two categories. This difference in the production of mottled yolks would tend to indicate that individual hens differ in their ability to absorb gossypol, or any other agent which might cause discolored yolks, from the feed. An analysis of the data using Friedman's rank test, described by Siegel (1956), showed a significant difference among hens in their ability to absorb gossypol from the feed. The test was significant at the 0.001 level of probability.

TABLE V

MOTTLING SCORES OF THE EGGS FROM TWO SELECTED HENS FROM RATION 1 ACCORDING TO STORAGE TEMPERATURES

Hen No.	Mottling Score	Temperature 1		Temperature 2	
		Number of Eggs	Per Cent	Number of Eggs	Per Cent
1	0	1	01.70	2	03.20
	1	6	10.00	4	06.40
	2	28	46.70	24	38.70
	3	25	41.70	32	51.60
13	0	57	95.00	56	90.32
	1	3	05.00	5	08.10
	2	0	00.00	1	01.60
	3	0	00.00	0	00.00

In an effort to detect gossypol in yolks of mottled eggs obtained from ration 1, an acetone powder was prepared from the mottled eggs and subjected to various tests. The results of the tests are shown in Table VI.

TABLE VI

RESULTS OF CHEMICAL TESTS OF ACETONE-EXTRACTED POWDER FROM MOTTLED EGG YOLKS FROM RATION 1

Chemical Test	Results
Ammonia	In comparison to the control ration, a dark color was produced.
Methyl-Ethyl Ketone and Oxalic Acid plus Aniline and Chloroform	An absorption spectrum gave no indication that gossypol was present.
Chloroform plus Antimony Tri-Chloride	An absorption spectrum gave no indication that gossypol was present.

Only the ammonia test, and it with only a slight indication, gave any evidence to indicate the presence of gossypol. However, some intact egg yolks when subjected to the ammonia test turned very dark, indicative of the presence of gossypol.

Eggs from ration 1 were removed from the shell and placed in a glass container with an air tight seal in an effort to determine visually if the amount of mottling increased under these conditions. No change in the yolks could be detected visually during a period of 14 days of storage at 2° C. Similar eggs were placed in a glass container and exposed to increased amounts of oxygen by bubbling air through the eggs. Again, no change was detected which would seem to indicate that the presence of mottling is not dependent upon oxygen. However, two eggs from hens known to produce severe mottling were sealed in paraffin and stored for 30 days. The eggs were then broken and examined for mottling. A normal yolk was found in one egg, while only slight mottling was present in the other. Both of the eggs turned dark when subjected to the ammonia test. This would seem to indicate that by

preventing the moisture loss and the gas exchange, usually occurring in stored eggs, the occurrence of mottling could be decreased.

Cottonseed Oil

The data in Table VII show the effects of the ration containing 5 per cent of cottonseed oil. Mottled yolks such as were found in eggs from ration 1 were found only in 0.12 per cent of the eggs from temperature 1 and 0.35 per cent of the eggs from temperature 2. However, yolk discolorations were in evidence in 14.52 per cent of the eggs from temperature 1 and 16.36 per cent of the eggs from temperature 2. These yolks appeared darker than normal or contained areas that were darker than the rest of the yolk. These dark areas were not the same as those abnormalities exhibited by the eggs from ration 1. In the comparison of the eggs from these two rations, yolk defects offer a better description for the abnormalities from ration 2 than does yolk mottling.

TABLE VII

PERCENTAGES OF EGG YOLK ABNORMALITIES OBSERVED AMONG EGGS PRODUCED FROM RATION 2

Abnormality	Temperature 1 (2° C.)	Temperature 2 (25° C.)
	Per Cent	
Mottled yolks	00.12	00.35
Blood spots	00.36	00.35
Viscous yolks	68.93	00.00
Other discolorations	14.52	16.36

Another defect was evidenced by the eggs from this ration. A condition described as viscous yolks was found in 68.93 per cent of the eggs from temperature 1. These yolks were often salmon colored. There was variation in color with some yolks having very light colored areas, seemingly just under the vitelline membrane, and others exhibiting the salmon color to differing degrees. Similar findings were reported by Evans et al. (1960). The egg yolks observed in the present study appeared very similar to those described by Shenstone and Vickery (1959) in which they described yolks to be very pasty or viscous, with some affected severely enough to be spherical when broken out on a flat plate. Eggs from the present study which were stored at 2° C. were very viscous and many fit the above description of being spherical and could be easily picked up without rupturing the vitelline membrane. The viscous yolks lost their abnormal viscosity when allowed to stand at room temperature for a short time.

The viscous yolks may be the result of increased amounts of saturated fatty acids caused by the ingestion of cottonseed oil. Evans et al. (1962) found this to be true in their studies. Increased saturated fatty acids would result in a high solidification point, which would explain the difference in the eggs from cold storage and the eggs from room temperature storage. Those eggs which were stored at 25° C. exhibited no viscosity as compared to those stored at 2° C.

The eggs from ration 2 were subjected to the same tests as those from ration 1 in an attempt to detect gossypol in the yolk. No evidence was found to indicate the presence of gossypol. When the egg yolks were subjected to the ammonia test, no color change was noted. This

would indicate that if gossypol were present the content in the yolks was very minute.

Piperazine

The results of feeding a ration containing piperazine, an anthelmintic, added at approximately the manufacturer's recommended level, are shown in Table VIII. Mottling from temperatures 1 and 2 was found in 0.12 and 0.46 per cent of the eggs, respectively. The occurrence was not of such a magnitude as to result in severe economic loss. The other defects were likewise not of a serious nature. Discolorations were observed in 6.34 per cent of the eggs stored at temperature 2 and in only 0.36 per cent of the eggs stored at temperature 1. This difference is believed to be due to storage temperature rather than the feed additive. These results concerning mottling of the egg yolks support findings reported by Fry and Wilson (1967).

TABLE VIII

PERCENTAGES OF EGG YOLK ABNORMALITIES OBSERVED AMONG EGGS PRODUCED FROM RATION 3

Abnormality	Temperature 1 (2° C.)	Temperature 2 (25° C.)
	Per Cent	
Mottled yolks	00.12	00.46
Blood spots	00.00	00.81
Viscous yolks	00.60	00.00
Other discolorations	00.36	06.34

Dibutyltin Dilaurate

Dibutyltine dilaurate, a compound used to prevent internal parasites, added at the manufacturer's recommended level, produced the results shown in Table IX. There was a low incidence of yolk mottling, with only 1.9 per cent of the eggs from temperature 1 and only 3.46 per cent of the eggs from temperature 2 showing the abnormality. Most of the abnormalities produced by ration 4 were classified as abnormal coloration. Discolorations were found in 26.43 per cent of the yolks from temperature 1 and 46.19 per cent of the yolks from temperature 2. These abnormalities varied somewhat in color and size, with the majority of the areas showing up somewhat darker than the normal yolk. Upon close examination many of the discolored areas appeared to be translucent and in some cases almost transparent.

TABLE IX

PERCENTAGES OF EGG YOLK ABNORMALITIES OBSERVED AMONG EGGS PRODUCED FROM RATION 4

Abnormality	Temperature 1 (2° C.)	Temperature 2 (25° C.)
	Per Cent	
Mottled yolks	01.90	03.46
Blood spots	05.95	05.53
Viscous yolks	00.60	00.00
Other discolorations	26.43	46.19

A slight increase in the occurrence of the discoloration was detected as the result of increased storage times at both storage temperatures, with the greatest increase being at the 25° C. As shown by Figure 4, the percentage of eggs showing darker areas tended to increase until day 13 at temperature 2 and day 14 at temperature 1, then decrease during the remainder of the storage period. No explanation as to the reason for the decrease toward the end of the storage periods has been found. Three storage periods (days 1, 21, and 22) were the only times when the number of abnormal eggs from temperature 1 exceeded the number from temperature 2. From this it might be concluded that the manifestation of these dark or translucent areas was increased as a result of the higher storage temperature.

It was also observed in ration 4, as well as in ration 1, that there was a large variation among hens. Of the 122 eggs produced by one hen, 68.03 per cent showed this discoloration, while another hen produced only 6.56 per cent of this type of abnormality.

It can be noted that a larger number of blood spots was found in eggs from ration 4 than from any of the other rations. However, this cannot be attributed to the dibutyltin dilaurate, since 56.12 per cent of the blood spots from both storage temperatures were produced by one individual hen. The hen in question produced 122 eggs, of which 55 (45%) were classified as having blood spots.

It is believed that all of the eggs classified under the category of dark or translucent areas would be objectionable to the consumer. As a result, the use of dibutyltin dilaurate in the layer ration could cause severe economic losses to the producer if fed over a period of time similar to that used in the present study. The overall period was

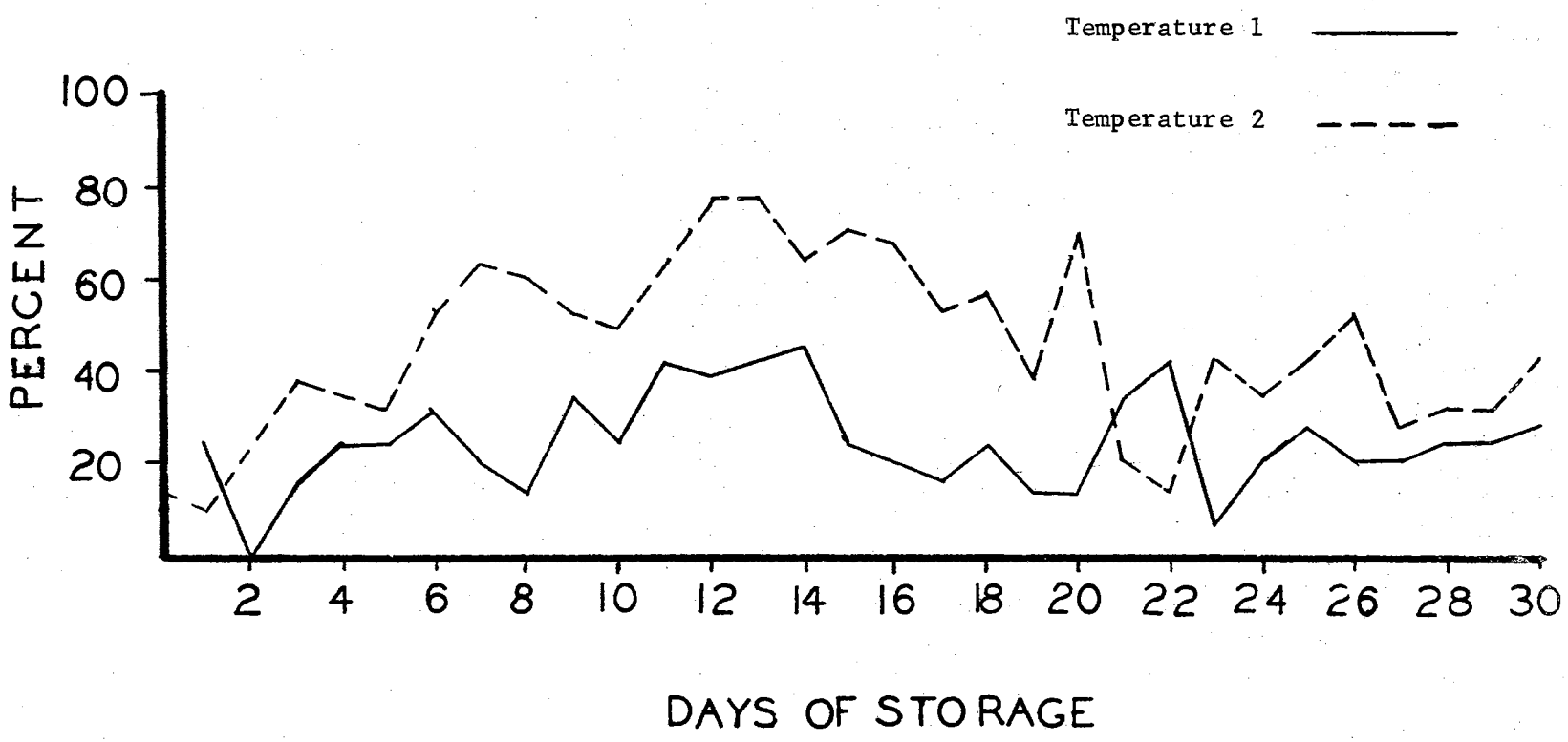

Figure 4. Percentages of Yolks Exhibiting Discolorations Other Than Mottling, Classified by Storage Temperatures

one production year, certainly much longer than the period recommended by the manufacturer.

Vegefat (Methyl Ester)

The results from the ration containing 5 per cent of vegefat are shown in Table X. Mottled yolks were found in 2.26 per cent of the eggs from temperature 1 and in 2.76 per cent of the eggs from temperature 2. Abnormalities at these levels would not result in a significant economic loss. The importance of lower storage temperatures is exhibited by the fact that 9.45 per cent of the eggs from temperature 2 and only 1.79 per cent of the eggs from temperature 1 showed evidence of discolorations in the yolk other than those classified as mottled. These discolorations, like those in the same category from other rations, were not the same as those classified as mottled.

TABLE X

PERCENTAGES OF EGG YOLK ABNORMALITIES OBSERVED AMONG EGGS PRODUCED FROM RATION 5

Abnormality	Temperature 1 (2° C.)	Temperature 2 (25° C.)
	Per Cent	
Mottled yolks	02.26	02.76
Blood spots	00.24	00.35
Viscous yolks	00.71	00.00
Other discolorations	01.79	09.45

Beef Tallow

The results of the ration containing 5 per cent of a special beef tallow are compiled in Table XI. Eggs from temperature 1 were found to contain 1.31 per cent mottled yolks and from temperature 2, 1.38 per cent mottled yolks. Mottling at these levels is not of such a magnitude as to be economically important. Other defects showed up at a lesser level, with the exception of discolored yolks from temperature 2. Of the total eggs, 2.88 per cent contained coloration which was not considered normal. However, this was not believed to be the result of the beef tallow in the diet, but rather the increased storage temperature, since all other rations produced a similar increase in discoloration at the higher temperature.

TABLE XI

PERCENTAGES OF EGG YOLK ABNORMALITIES OBSERVED AMONG EGGS PRODUCED FROM RATION 6

Abnormality	Temperature 1 (2° C.)	Temperature 2 (25° C.)
	Per Cent	
Mottled yolks	01.31	01.38
Blood spots	00.95	00.35
Viscous yolks	00.00	00.00
Other discolorations	00.83	02.88

The beef tallow incorporated into the ration was secured from an animal which was fed a high level of cottonseed products. A positive halphen test on the tallow indicated the probable presence of an acid such as sterculic or malvalic as result of the ingestion of cottonseed

oil which contained such acids. The presence of such acids in the diet of laying hens has been shown to cause abnormal yolks (Shenstone and Vickery, 1959). The abnormality was suggested to be the result of a high level of saturated fatty acids present in the egg yolks. The author was not able to demonstrate such abnormalities during the course of the present study.

Gossypol

Pure gossypol, incorporated into the layer ration at the 0.003 per cent level, produced the results shown in Table XII. An examination of the eggs showed that 1.31 per cent from temperature 1 and 0.81 per cent from temperature 2 were mottled. This was not enough mottling to be considered significant. No other defects were found in quantities that would be economically important, with the possible exception of the yolk discolorations from temperature 2. Those defective eggs, 5.41 per cent of the total, were believed to be the result of the higher storage temperature rather than the result of the added gossypol, since results from all tested ingredients showed an increase in discolored yolks at temperature 2.

TABLE XII

PERCENTAGES OF EGG YOLK ABNORMALITIES OBSERVED AMONG EGGS PRODUCED FROM RATION 8

Abnormality	Temperature 1 (2° C.)	Temperature 2 (25° C.)
	Per Cent	
Mottled yolks	01.31	00.81
Blood spots	00.83	01.27
Viscous yolks	00.36	00.00
Other discolorations	00.60	05.41

CHAPTER V

SUMMARY AND CONCLUSIONS

The purpose of this study was to evaluate the effect of selected feed ingredients and additives on the incidence of egg yolk abnormalities during storage. The materials tested included cottonseed meal, cottonseed oil, piperazine, dibutyltin dilaurate, special beef tallow, vegefat, and pure gossypol. The storage period extended over 30 days, with daily observations being made. In order to develop the subject more adequately, two storage temperatures were used, the normal egg storage temperature (2° C.) and room temperature (25° C.). A total of 13,664 eggs were examined throughout the study.

The data were analyzed and the following conclusions can be made from the analysis:

1. Cottonseed meal incorporated at the 9 per cent level in the layer ration produced olive to brown colored yolks, and in some cases almost black yolks. Yolk mottling varied greatly among hens, which would indicate differences in the ability of individual hens to absorb the causative agent, gossypol, from the feed. No increase in mottling due to storage could be detected when eggs were examined on a day-to-day basis for storage periods ranging from one to thirty days.

2. Cottonseed oil incorporated in the layer ration at a level of 5 per cent failed to produce eggs that showed abnormalities which could be classified as mottled yolks. However, salmon colored yolks which were very viscous were in evidence. The increased viscosity of these yolks was believed to have been the result of increased amounts of saturated fatty acids being deposited in the egg yolk due to ingestion of cottonseed oil by the hens. Increased amounts of saturated fatty acids would produce a high solidification point, which would account for the fact that eggs stored at 25° C. failed to exhibit the defect. Some of the salmon yolks showed white areas resembling coagulated albumen just under the vitelline membrane. It is not know what might have caused these areas to be present. In addition, a large number of eggs showed areas that were darker than would be considered normal. The eggs described as being abnormal would be objectionable to consumers.
3. Piperazine, added to the ration at the manufacturer's recommended level, failed to produce yolk defects to an extent significantly different from the control ration.
4. The use of dibutyltin dilaurate at the level recommended by the manufacturer produced eggs which would be objectionable from a consumer standpoint. The eggs had dark areas on the yolks, some of which seemed almost transparent. Storage temperature influenced the occurrence of abnormalities, with the most frequent occurrence in the eggs stored at 25° C. It was noted that the discolorations seemed to increase with increased storage time until about midway through the 30-day storage

period and then decreased toward the end of the storage period. No immediate explanation as to the reason for the increase and the later decrease has been found. A difference among hens was observed in the manifestation of these discolorations.

5. The use of vegefat (methyl ester) in the layer ration caused no noticeable changes in the yolks of stored eggs.
6. Beef tallow from an animal which had received a high percentage of cottonseed products had no detrimental effect on yolk color.
7. Pure gossypol at the 0.003 per cent level was found to cause no mottling in either the fresh or stored eggs.
8. Storage at the higher temperature (25° C.) resulted in increased discolorations in eggs from all rations tested. These discolorations could not be classified in the same category as the mottled eggs produced by cottonseed meal, and might better be referred to as yolk defects.

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APPENDICES

APPENDIX A

COMPOSITION OF EXPERIMENTAL BASAL

Ingredient	Per Cent
Corn, ground yellow	28.4
Milo, ground	27.5
Wheat bran	5.0
Oat mill feed	5.0
Soybean oil meal (50% protein)	1.0
Fish meal (60% protein)	3.8
Meat and bone scrap (50% protein)	3.8
Blood meal (80% protein)	0.6
Alfalfa meal (17% protein)	1.3
Whey, dried	1.3
Yeast culture	1.3
dl-Methionine	0.1
Dicalcium phosphate (18% P, 32% Ca)	4.0
Calcium carbonate (35% Ca)	1.9
VMC-60*	0.5
Salt	0.5
Ingredients to be added later	14.0
Total	100.0

*0.5 per cent of VMC-60 adds the following per lb. of finished diet: Vitamin A, 8,000 U.S.P. units; Vitamin D_3 1,200 I.C.U.; Vitamin E, 6 I.U.; Vitamin K, 3 mg.; Vitamin B_{12}, 0.008 mg.; Riboflavin, 4 mg.; Niacin, 32 mg.; Pantothenic acid, 8 mg.; Choline chloride, 500 mg.; Manganese, 27.7 mg.; Iodine, 0.86 mg.; Cobalt, 0.59 mg.; Iron, 21.8 mg.; Copper, 1.65 mg.; Zinc, 22.7 mg.

APPENDIX B

COMPOSITION OF EXPERIMENTAL RATIONS

Ingredient	Ration Number							
	1	2	3	4	5	6	7	8
	Per Cent							
Experimental basal	86.0	86.0	86.0	86.0	86.0	86.0	86.0	86.0
Soybean oil mean (50% protein)		7.9	7.9	7.9	7.9	7.9	7.9	7.9
Corn oil	5.0		5.0	5.0			5.0	5.0
Corn, ground yellow		1.1	1.1	1.1	1.1	1.1	1.1	1.1
Cottonseed oil		5.0						
Cottonseed meal	9.0							
Vegefat (methyl ester)					5.0			
Special beef tallow						5.0		
Pure gossypol								(1)*
Piperazine			(2)*					
Dibutyltin dilaurate				(3)*				

*1 Pure gossypol added at the rate of 1.326 gm./100 lb.

2 Piperazine added at the rate of 25 gm./100 lb.

3 Dibutyltin dilaurate added at the rate of 31.78 gm./100 lb.

VITA

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